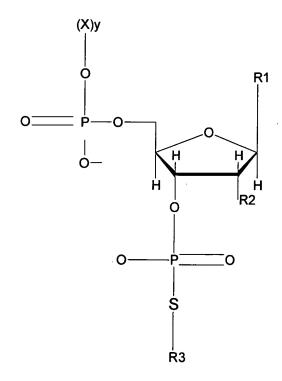
Serial No.: 09/882,274 Filed: June 15, 2001

Page 2

CURRENT STATUS OF ALL CLAIMS

1. (Currently amended) A method of non-enzymatic ligation of a nucleic acid, comprising contacting a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide under conditions that allow formation of a phosphodiester bond between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, whereby a ligated nucleic acid product is generated.

2. (Previously amended) The method of claim 1, wherein said polynucleotide-3' phosphorothiolate comprises a moiety having the formula:



Serial No.: 09/882,274 Filed: June 15, 2001

Page 3

wherein,

X is a nucleotide;

y is a positive integer;

R1 is a nucleotide base;

R2 is a hydrogen atom or hydroxyl; and

R3 is nitrophenyl.

- 3. (Original) The method of claim 1, wherein said polynucleotide-3' phosphorothiolate further comprises a duplex polynucleotide.
- 4. (Original) The method of claim 1, wherein said acceptor polynucleotide further comprises a duplex polynucleotide.
- 5. (Amended) A method of <u>replicating a ligated</u>
 <u>nucleic acid product</u> generating a polynucleotide product,
 comprising:
- (a) contacting a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide under conditions that allow formation of a phosphodiester bond between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, wherein a polynucleotide-3' phosphorothiolate having a phosphodiester bond with said acceptor polynucleotide is formed phosphodiester bond is formed between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide to generate a ligated nucleic acid product, wherein one of said polynucleotide-3' phosphorothiolate or said acceptor polynucleotide comprises a vector, and

Serial No.: 09/882,274 Filed: June 15, 2001

Page 4

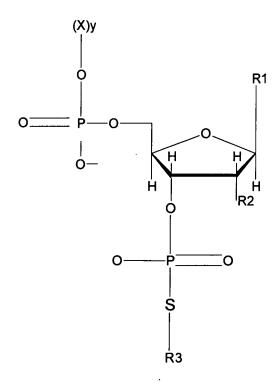
- (b) transducing into a host cell said polynucleotide product, wherein said polynucleotide product is replicated in said host cell polynucleotide-3' phosphorothiclate having a phosphodiester bond with said acceptor polynucleotide.
- 6. (Currently amended) A method of non-enzymatic ligation of a nucleic acid, comprising:
- (a) contacting a polynucleotide-3' phosphorothiolate precursor and an activator under conditions sufficient to react said polynucleotide-3' phosphorothiolate precursor and said activator, wherein said polynucleotide-3' phosphorothiolate precursor reacts with said iodonitrobenzene to produce a polynucleotide-3' phosphorothiolate, and
- (b) contacting said polynucleotide-3' phosphorothiolate with an acceptor polynucleotide under conditions that allow formation of a phosphodiester bond between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, whereby a ligated nucleic acid product is generated.
- 7. (Original) The method of claim 6, wherein said activator is iodonitrobenzene.
- 8. (Currently amended) A method of molecular eloning ligating a vector and an insert comprising, contacting an insert comprising a polynucleotide-3' phosphorothicate with an acceptor vector under conditions that allow formation of a phosphodiester bond between said insert and said acceptor

Serial No.: 09/882,274 Filed: June 15, 2001

Page 5

vector, wherein a phosphodiester bond is formed between said insert and said acceptor vector, whereby a ligated product vector comprising said insert is generated to generate a vector comprising an insert polynucleotide.

- 9. (Currently amended) The method of claim 8, further comprising transforming said vector comprising said insert an insert polynucleotide into a host cell.
- 10. (Previously amended) The method of claim 8, wherein said polynucleotide-3' phosphorothiolate comprises a moiety having the formula:



wherein,

X is a nucleotide;

y is a positive integer;

Serial No.: 09/882,274 Filed: June 15, 2001

Page 6

R1 is a nucleotide base;

R2 is a hydrogen atom or hydroxyl; and R3 is nitrophenyl.

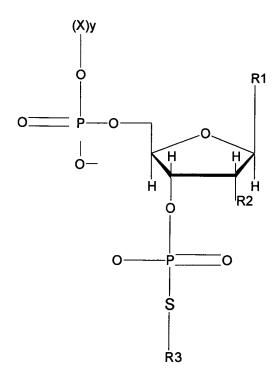
- 11. (Currently amended) A method of molecular eloning ligating a vector and an insert comprising:
- (a) contacting a polynucleotide-3' phosphorothiolate precursor and iodonitrobenzene under conditions sufficient to react said polynucleotide-3' phosphorothiolate precursor and said iodonitrobenzene, wherein said polynucleotide-3' phosphorothiolate precursor reacts with said iodonitrobenzene to produce a polynucleotide-3' phosphorothiolate, and
- (b) contacting an insert comprising said polynucleotide-3' phosphorothiolate with an acceptor vector under conditions that allow formation of a phosphodiester bond between said insert and said acceptor vector, wherein a phosphodiester bond is formed between said insert and said acceptor vector, whereby a ligated product vector comprising said insert is generated to generate a vector comprising an insert polynucleotide.
- 12. (Currently amended) A method of molecular eloning ligating a vector and a polynucleotide comprising, contacting a vector comprising a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide, under conditions that allow formation of a phosphodiester bond between said vector and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said vector and said acceptor polynucleotide, whereby a ligated product vector

Serial No.: 09/882,274 Filed: June 15, 2001

Page 7

comprising said polynucleotide is generated to generate a vector comprising said acceptor polynucleotide.

- 13. (Original) The method of claim 12, further comprising transforming said vector comprising said acceptor polynucleotide into a host cell.
- 14. (Previously amended) The method of claim 12, wherein said polynucleotide-3' phosphorothiolate comprises a moiety having the formula:



wherein,

X is a nucleotide;

y is a positive integer;

R1 is a nucleotide base;

Serial No.: 09/882,274 Filed: June 15, 2001

Page 8

R2 is a hydrogen atom or hydroxyl; and R3 is nitrophenyl.

- 15. (Currently amended) The method of claim 12, wherein said vector further comprises a 3' phosphorothiolate moiety at one or more terminal ends of said vector.
- 16. (Currently amended) A method of <u>ligating a</u> vector and a polynucleotide molecular cloning comprising:
- (a) contacting a polynucleotide-3' phosphorothiolate precursor and an activator under conditions sufficient to react said polynucleotide-3' phosphorothiolate precursor and said activator to produce a polynucleotide-3' phosphorothiolate, wherein said polynucleotide-3' phosphorothiolate precursor reacts with said iodonitrobenzene to produce a polynucleotide-3' phosphorothiolate, and
- (b) contacting a vector comprising said polynucleotide-3' phosphorothiolate with an acceptor polynucleotide, under conditions that allow formation of a phosphodiester bond between said vector and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said vector and said acceptor polynucleotide, whereby a ligated product vector comprising said polynucleotide is generated to generate a vector comprising said acceptor polynucleotide.

Claims 17 to 56. Cancelled previously.